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(54) Title: POLYPEPTIDES WITH INTERLEUKIN-8 RECEPTOR BINDING

(57) Abstract

The present invention presents sequences of polypeptides capable of modulating IL8 receptor binding and IL8 receptor-mediated biological response. Also, polynucleotides encoding the instant polypeptides and methods of producing the polypeptides are also described.

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# Polypeptides with Interleukin-8 Receptor Binding

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## Description

### Technical Field

The invention relates generally to IL8 mutants capable of binding to IL8 receptor 1 (IL8R1) or IL8 receptor 2 (IL8R2). The polypeptides of the present invention can be used to inhibit IL8 receptor binding. Further, the polypeptides can be used as either agonists or antagonists to IL8.

### Background of the Invention

Cells utilize diffusible mediators, called cytokines, to signal one another. A superfamily of cytokines are the chemokines, which includes IL8. A review article of the chemokine superfamily was written by Miller et al., Crit Rev Immun 12(1,2): 17-46 (1992) and by Baggiolini et al. Adv Immunol 55: 97-179 (1994), herein incorporated by reference.

Native human IL8 acts as a chemoattractant for neutrophils, and induces granulocytosis upon systemic injection and skin reaction upon local injection in experimental animals. See Bazzoni et al., (1991) 173: 771-774; Van Damme et al., J Exp Med 167: 1364-1376; Ribero et al., Immunology 73: 472-477 (1991). The molecule also activates the release of superoxide anions and elicits release of primary granule constituents of neutrophils, including myeloperoxidase, β-glucuronidase, and elastase. Native human

IL8 mediates these biological activities by binding to its receptor and triggering transduction, a cascade of reactions ultimately resulting in a biological response.

Presently, two IL8 binding receptors have been identified and are termed "IL8R1" and "IL8R2." The amino acid sequence of these polypeptides are described in Murphy et al., Science 253: 1280 (1991) and Holmes et al., Science 253: 1278 (1991), herein incorporated by reference. Other proteins can compete with IL8 to bind to IL8R2, such as GROα, GROβ, and GROγ. NAP-2 AND ENA-78 have been implicated with IL8R2 binding by cross-desensitization experiments with native IL8 by measuring $Ca^{2+}$.

These other proteins which can compete for IL8 binding are members of the chemokine family. The chemokines are a group of structurally and functionally related cytokines. Recent studies indicated that these proteins function in the recruitment and activation of leukocytes and other cells at sites of inflammation and, therefore, appear to be important inflammatory mediators.

Disclosure of the Invention

The object of the invention provides the following polypeptides with altered IL8 receptor binding characteristics compared to native human IL8:

R47K: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine, SEQ ID NO:2;

L49A: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to alanine, SEQ ID NO:3;

E48K, L49A: exhibiting the same amino acid sequence as native human IL8 except position 48 is changed from glutamic acid to lysine and position 49 is changed from leucine to alanine, SEQ ID NO:4;

E48K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 48 is changed from glutamic acid to lysine and position 52 is changed from aspartic acid to asparagine, SEQ ID NO:5;

R47K, E48K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine, position 48 is changed from glutamic acid to lysine and position 52 is changed from aspartic acid to asparagine, SEQ ID NO:6;

R47K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine and position 52 is changed from aspartic acid to asparagine, SEQ ID NO:7;

Y13L: exhibiting the same amino acid sequence as native human IL8 except position 13 is changed from tyrosine to leucine, SEQ ID NO:8;

S14Q: exhibiting the same amino acid sequence as native human IL8 except position 14 is changed from serine to glutamine, SEQ ID NO:9;

V41F: exhibiting the same amino acid sequence as native human IL8 except position 41 is changed from valine to phenylalanine, SEQ ID NO:10;

D45R: exhibiting the same amino acid sequence as native human IL8 except position 45 is changed from aspartic acid to arginine, SEQ ID NO:11;

L49F: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to phenylalanine, SEQ ID NO:12;

L49S: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to serine, SEQ ID NO:13;

F21N: exhibiting the same amino acid sequence as native human IL8 except position 21 is changed from phenylalanine to asparagine, SEQ ID NO:14; and

Y13L/S14Q: exhibiting the same amino acid sequence as native human IL8 except position 13 is changed from a tyrosine to a leucine and position 14 is changed from a serine to a glutamine, SEQ ID NO:15.

Another object of the invention is to provide mutants, fragment, and fusions of the following reference polypeptides:

R47K: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine, wherein position 47 is not changed from lysine in the amino acid sequence of the mutants, fragments, or fusions;

L49A: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to alanine, wherein position 49 is not changed from alanine in the amino acid sequence of the mutants, fragments, or fusions;

E48K, L49A: exhibiting the same amino acid sequence as native human IL8 except position 48 is changed from glutamic acid to lysine and position 49 is changed from

leucine to alanine, wherein position 48 is not changed from lysine and position is not changed from alanine in the amino acid sequence of the mutants, fragments, or fusions;

E48K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 48 is changed from glutamic acid to lysine and position 52 is changed from aspartic acid to asparagine, wherein position 48 is not changed from lysine and position 52 is not changed from asparagine in the amino acid sequence of the mutants, fragments, or fusions;

R47K, E48K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine, position 48 is changed from glutamic acid to lysine and position 52 is changed from aspartic acid to asparagine, wherein position 47 is not changed from lysine, position 48 is not changed from lysine and position 52 is not changed from asparagine in the amino acid sequence of the mutants, fragments, or fusions;

R47K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine and position 52 is changed from aspartic acid to asparagine, wherein position 47 is not changed from lysine and position 52 is not changed from asparagine in the amino acid sequence of the mutants, fragments, or fusions;

Y13L: exhibiting the same amino acid sequence as native human IL8 except position 13 is changed from tyrosine to leucine, wherein position 13 is not changed from leucine in the amino acid sequence of the mutants, fragments, or fusions;

S14Q: exhibiting the same amino acid sequence as native human IL8 except position 14 is changed from serine to glutamine, wherein position 14 is not changed from glutamine in the amino acid sequence of the mutants, fragments, or fusions;

V41F: exhibiting the same amino acid sequence as native human IL8 except position 41 is changed from valine to phenylalanine, wherein position 41 is not changed from phenylalanine in the amino acid sequence of the mutants, fragments, or fusions;

D45R: exhibiting the same amino acid sequence as native human IL8 except position 45 is changed from aspartic acid to arginine, wherein position 45 is not changed from arginine in the amino acid sequence of the mutants, fragments, or fusions;

L49F: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to phenylalanine, wherein position 49 is not changed from phenylalanine in the amino acid sequence of the mutants, fragments, or fusions;

L49S: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to serine, wherein position 49 is not changed from serine in the amino acid sequence of the mutants, fragments, or fusions;

F21N: exhibiting the same amino acid sequence as native human IL8 except position 21 is changed from phenylalanine to asparagine, wherein position 21 is not changed from asparagine in the amino acid sequence of the mutants, fragments, or fusions; and

Y13L/S14Q: exhibiting the same amino acid sequence as native human IL8 except position 13 is changed from tyrosine to leucine and position 14 is changed from serine to glutamine, wherein position 13 is not changed from leucine and position 14 is not changed from glutamine in the amino acid sequence of the mutants, fragments, or fusions.

Yet another object of the invention is a method of inhibiting receptor binding of native IL8 comprising:

(a) providing a polypeptide with altered IL8 receptor binding;

(b) contacting the receptor with an effective inhibiting amount of the polypeptide.

Also, another object of the invention is a method of modulating an IL8 receptor-mediated biological response comprising:

(a) providing an polypeptide with altered IL8 receptor binding;

(b) contacting a cell that produces an IL8 receptor with an effective modulating amount of the polypeptide.

Modes of Carrying Out The Invention

A. Definitions

A "native IL8 polypeptide" refers to a polypeptide which is identical to a sequence recovered from a source which naturally produces IL8, such as human, bovine, porcine or other mammalian sources. Native IL8 may be vary in length from species to species. An example of native IL8 is the native human IL8 which has the amino acid sequence shown in SEQ ID NO:1.

Reference polypeptides refer to polypeptides with the following the amino acid sequences:

R47K: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine, SEQ ID NO:2;

L49A: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to alanine, SEQ ID NO:3;

E48K, L49A: exhibiting the same amino acid sequence as native human IL8 except position 48 is changed from glutamic acid to lysine and position 49 is changed from leucine to alanine, SEQ ID NO:4;

E48K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 48 is changed from glutamic acid to lysine and position 52 is changed from aspartic acid to asparagine, SEQ ID NO:5;

R47K, E48K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine, position 48 is changed from glutamic acid to lysine and position 52 is changed from aspartic acid to asparagine, SEQ ID NO:6;

R47K, D52N: exhibiting the same amino acid sequence as native human IL8 except position 47 is changed from arginine to lysine and position 52 is changed from aspartic acid to asparagine, SEQ ID NO:7;

Y13L: exhibiting the same amino acid sequence as native human IL8 except position 13 is changed from tyrosine to leucine, SEQ ID NO:8;

S14Q: exhibiting the same amino acid sequence as native human IL8 except position 14 is changed from serine to glutamine, SEQ ID NO:9;

V41F: exhibiting the same amino acid sequence as native human IL8 except position 41 is changed from valine to phenylalanine, SEQ ID NO:10;

D45R: exhibiting the same amino acid sequence as native human IL8 except position 45 is changed from aspartic acid to arginine, SEQ ID NO:11;

L49F: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to phenylalanine, SEQ ID NO:12;

L49S: exhibiting the same amino acid sequence as native human IL8 except position 49 is changed from leucine to serine, SEQ ID NO:13;

F21N: exhibiting the same amino acid sequence as native human IL8 except position 21 is changed from phenylalanine to asparagine, SEQ ID NO:14; and

Y13L/S14Q: exhibiting the same amino acid sequence as native human IL8 except position 13 is changed from a tyrosine to a leucine and position 14 is changed from a serine to a glutamine, SEQ ID NO: 15.

"Mutants" are polypeptides that contain amino acid substitutions, deletions, or insertions compared to the reference polypeptides. Mutants of the reference polypeptides having an amino acid sequence which retain at least 80% amino acid sequence identity with a reference polypeptide; more typically, at least 85%; even more typically, at least 90%. Preferably mutants will retain at least 92% amino acid sequence identity with a reference polypeptide; more preferably, at least 95%; even more preferably, at least 98%. Further, the mutants of the reference polypeptides will retain at least 50% receptor binding or biological activity with a reference polypeptide; more typically, at least 60%; even more typically, at least 75%. Preferably mutants will retain at least 80% receptor binding or biological activity with a reference polypeptide; more preferably, at least 85%; even more preferably, at least 90%; even more preferably, at least 95%.

"Fragments" possess the same amino acid sequence of the mutants or reference except the fragments lack the amino and/or carboxyl terminal sequences of the reference polypeptides. The number of amino acids that are truncated is not critical as long as the fragment retains at least 50% receptor binding or biological activity of a reference polypeptide; more typically, at least 60%; even more typically, at least 75%. Preferably fragments will retain at least 80% receptor binding or biological activity of a reference polypeptide; more preferably, at least 85%; even more preferably, at least 90%; even more

preferably, at least 95%. Further, fragments will retain at least 80% amino acid sequence identity with a reference polypeptide; more typically, at least 85%; even more typically, at least 90%. Preferably fusions will retain at least 92% amino acid sequence identity with a reference polypeptide; more preferably, at least 95%; even more preferably, at least 98%.

"Fusions" are mutants, fragments, or the reference polypeptides that also include amino and/or carboxyl terminal amino acid extensions. The fusions, just as the mutants, fragments, retain at least 50% receptor binding or biological activity of a reference polypeptide; more typically, at least 60%; even more typically, at least 75%. Preferably fusions will retain at least 80% receptor binding or biological activity of a reference polypeptide; more preferably, at least 85%; even more preferably, at least 90%; even more preferably, at least 95%. Further, fusions will retain at least 80% amino acid sequence identity with a reference polypeptide; more typically, at least 85%; even more typically, at least 90%. Preferably fusions will retain at least 92% amino acid sequence identity with a reference polypeptide; more preferably, at least 95%; even more preferably, at least 98%.

By "modulating an IL8 receptor-mediated biological response" is meant either increasing or decreasing the incidence of one or more cellular activities normally triggered by the binding of IL8 to its receptor. The nature of these activities may be biochemical or biophysical. For example, a substance would "modulate an IL8 receptor-mediated biological response" if it does not stimulated the same signal transduction activity as IL8 when the polypeptides of the instant invention binds to an IL8 receptor.

More particularly, a cascade of biochemical reactions is triggered when IL8 binds to its receptor. Accordingly, an IL8 inhibitor will "modulate an IL8 receptor-mediated biological response" when it causes an increase or decrease in any one of these reactions. Other biological activities attributable to IL8 which can be measured in order to determine modulation include, for example, neutrophil chemotactic activity, methaseued using assays described in Schroder et al., J Immunol. 139: 3474-3483 (1987). Also, IL8 has been implicated in rapid mobilization of hematopoietic stem cells (Laterveer, et al. Blood 85: 8 2269-2275 (1995)) and implicated signal transduction in T-lymphocytes (Bacon et al., J Immunology 154: 3654-3666 (1995)).

An "effective inhibiting amount" of the polypeptides of the instant invention refers to an amount sufficient to block the binding, in whole or in part, of native IL8 to an

IL8 receptor. Typically, an effective amount inhibits at least 20% of the native IL8 receptor binding. More typically, the polypeptides inhibit at least 40%, even more typically the polypeptides inhibit at least 60% of the native IL8 receptor binding; most preferably at least 70%.

The term "effective modulating amount" or a polypeptide of the instant invention refers to an amount sufficient to cause a change in an IL8 receptor-mediated biological activity, as described above. Typically, an effective amount causes a change at least 20% compared to the response to native IL8 receptor-mediated biological response. More typically, the polypeptides cause a change of least 40%, even more typically at least 60% of the native IL8 receptor binding; most preferably at least 100%.

B. General Method

The following reference polypeptides have altered IL8 receptor binding characteristics as compared with native human IL8. Such polypeptides, as well as mutants, fragments, and fusions of these polypeptides, can be produced by synthesizing the desired amino acid sequence and refolding the polypeptide. See, for example, Clark-Lewis et al., J Biol Chem 269(23): 16075-1081 (1994).

The reference polypeptides with altered IL8 receptor binding characteristics, and mutants, fragment, and fusions of such, can also be constructed utilizing Produced by recombinant methods.

Using recombinant methods, a coding sequence for the desired polypeptide can be constructed synthetically or by altering the coding sequence of native IL8 polypeptides. For example, the coding sequence of native IL8 polypeptides can be determined by screening libraries using probes based on published sequences. Alternatively, synthetic genes can be made using codons preferred by the host cell to encode the desired polypeptide. See Urdea *et al.*, Proc. Natl. Acad. Sci. USA 80: 7461 (1983).

The native IL8 sequences can be altered to construct the reference sequences or the mutants, fragments, or fusions of the reference polypeptides. For example, mutants can be created by making conservative amino acid substitutions. The following are examples of conservative substitutions: Gly ↔ Ala; Val ↔ Ile ↔ Leu; Asp ↔ Glu; Lys ↔ Arg; Asn ↔ Gln; and Phe ↔ Trp ↔ Tyr. Mutants can also contain amino acid deletions or insertions compared to the reference polypeptides.

Mutants of the reference polypeptides having an amino acid sequence which retain at least 80% amino acid sequence identity with a reference polypeptide; more typically, at least 85%; even more typically, at least 90%. Preferably mutants will retain at least 92% amino acid sequence identity with a reference polypeptide; more preferably, at least 95%; even more preferably, at least 98%.

Fragments possess the same amino acid sequence of the mutants or reference except the fragments lack the amino and/or carboxyl terminal sequences of the reference polypeptides. The number of amino acids that are truncated is not critical as long as the fragment retains at least 70% of the IL8 receptor binding of a reference polypeptide; more typically, at least 75%; even more typically, at least 80%. Preferably mutants will retain at least 85% amino acid sequence identity with a reference polypeptide; more preferably, at least 90%. The coding sequence of such fragments can be easily constructed by cleaving the unwanted nucleotides from the mutant or reference polypeptide coding sequences.

Fusions are mutants, fragments, or the reference polypeptides that also include amino and/or carboxyl terminal amino acid extensions. The fusions, just as the mutants, fragments, retain at least 70% of the IL8 receptor binding of a reference polypeptide; more typically, at least 75%; even more typically, at least 80%. Preferably mutants will retain at least 85% amino acid sequence identity with a reference polypeptide; more preferably, at least 90%. Coding sequence of the fusions can be constructed by ligating synthetic polynucleotides encoding the additional amino acids to fragment, mutant, or reference polypeptide coding sequences.

Production of IL8R1 Binding Compounds by Host Cells

At the minimum, an expression vector will contain a promoter which is operable in the host cell and operably linked to the desired coding sequence. Expression vectors may also include signal sequences, terminators, selectable markers, origins of replication, and sequences homologous to host cell sequences. These additional elements are optional but can be included to optimize expression.

A promoter is a DNA sequence upstream or 5' to the desired coding sequence to be expressed. The promoter will initiate and regulate expression of the coding sequence in the desired host cell. To initiate expression, promoter sequences bind RNA polymerase and initiate the downstream (3') transcription of a coding sequence (*e.g.* structural gene) into mRNA. A promoter may also have DNA sequences that regulate the rate of expression by enhancing or specifically inducing or repressing transcription. These sequences can overlap the sequences that initiate expression. Most host cell systems include regulatory sequences within the promoter sequences. For example, when a repressor protein binds to the lac operon, an *E. coli* regulatory promoter sequence, transcription of the downstream gene is inhibited. Another example is the yeast alcohol dehydrogenase promoter, which has an upstream activator sequence (UAS) that modulates expression in the absence of a readily available source of glucose. Additionally, some viral enhancers not only amplify but also regulate expression in mammalian cells. These enhancers can be incorporated into mammalian promoter sequences, and the promoter will become active only in the presence of an inducer, such as a hormone or enzyme substrate (Sassone-Corsi and Borelli (1986) Trends Genet. 2:215; Maniatis *et al.* (1987) Science 236:1237).

Functional non-natural promoters may also be used, for example, synthetic promoters based on a consensus sequence of different promoters. Also, effective promoters can contain a regulatory region linked with a heterologous expression initiation region. Examples of hybrid promoters are the *E. coli* lac operator linked to the *E. coli* tac transcription activation region; the yeast alcohol dehydrogenase (ADH) regulatory sequence linked to the yeast glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) transcription activation region (U.S. Patent Nos. 4,876,197 and 4,880,734, incorporated herein by reference); and the cytomegalovirus (CMV) enhancer linked to the SV40 (simian virus) promoter.

The desired coding sequence may also be linked in reading frame to a signal sequence. The signal sequence fragment typically encodes a peptide comprised of hydrophobic amino acids which directs the desired polypeptide to the cell membrane. Preferably, there are processing sites encoded between the leader fragment and the gene or fragment thereof that can be cleaved either *in vivo* or *in vitro*. DNA encoding suitable signal sequences can be derived from genes for secreted endogenous host cell proteins, such as the yeast invertase gene (EP 12 873; JP 62,096,086), the A-factor gene (U.S. Patent No. 4,588,684), interferon signal sequence (EP 60 057).

A preferred class of secretion leaders, for yeast expression, are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (typically about 25 to about 50 amino acid residues) (U.S. Patent Nos. 4,546,083 and 4,870,008, incorporated herein by reference; EP 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast signal sequence, but a pro-region from a second yeast alpha-factor. (See *e.g.*, PCT WO 89/02463.)

Typically, terminators are regulatory sequences, such as polyadenylation and transcription termination sequences, located 3' or downstream of the stop codon of the coding sequences. Usually, the terminator of native host cell proteins are operable when attached 3' of the desired coding sequences. Examples are the *Saccharomyces cerevisiae* alpha-factor terminator and the baculovirus terminator. Further, viral terminators are also operable in certain host cells; for instance, the SV40 terminator is functional in CHO cells.

For convenience, selectable markers, an origin of replication, and homologous host cells sequences may optionally be included in an expression vector. A selectable marker can be used to screen for host cells that potentially contain the expression vector. Such markers may render the host cell immune to drugs such as ampicillin, chloramphenicol, erythromycin, neomycin, and tetracycline. Also, markers may be biosynthetic genes, such as those in the histidine, tryptophan, and leucine pathways. Thus,

when leucine is absent from the media, for example, only the cells with a biosynthetic gene in the leucine pathway will survive.

An origin of replication may be needed for the expression vector to replicate in the host cell. Certain origins of replication enable an expression vector to be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the 2μ and autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

Expression vectors may be integrated into the host cell genome or remain autonomous within the cell. Polynucleotide sequences homologous to sequences within the host cell genome may be needed to integrate the expression cassette. The homologous sequences do not always need to be linked to the expression vector to be effective. For example, expression vectors can integrate into the CHO genome via an unattached dihydrofolate reductase gene. In yeast, it is more advantageous if the homologous sequences flank the expression cassette. Particularly useful homologous yeast genome sequences are those disclosed in PCT WO90/01800, and the HIS4 gene sequences, described in Genbank, accession no. J01331.

The choice of promoter, terminator, and other optional elements of an expression vector will also depend on the host cell chosen. The invention is not dependent on the host cell selected. Convenience and the level of protein expression will dictate the optimal host cell. A variety of hosts for expression are known in the art and available from the American Type Culture Collection (ATCC). Bacterial hosts suitable for expressing the desired polypeptide include, without limitation: *Campylobacter*, *Bacillus*, *Escherichia*, *Lactobacillus*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus*. Yeast hosts from the following genera may be utilized: *Candida*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, and *Yarrowia*. Immortalized mammalian host cells include but are not limited to CHO cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and other cell lines. A number of insect cell hosts are also available for expression of heterologous proteins: *Aedes aegypti*, *Bombyx mori*, *Drosophila melanogaster*, and *Spodoptera frugiperda* (PCT WO 89/046699; Carbonell *et al.*, (1985) J. Virol. 56:153;

Wright (1986) Nature 321:718; Smith *et al.*, (1983) Mol. Cell. Biol. 3:2156; and see generally, Fraser, *et al.* (1989) In Vitro Cell. Dev. Biol. 25:225).

Transformation

After vector construction, the desired polypeptide expression vector is inserted into the host cell. Many transformation techniques exist for inserting expression vectors into bacterial, yeast, insect, and mammalian cells. The transformation procedure to introduce the expression vector depends upon the host to be transformed.

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and typically protocol includes either treating the bacteria with $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation or viral infection. Transformation procedures usually vary with the bacterial species to be transformed. See *e.g.*, (Masson *et al.* (1989) FEMS Microbiol. Lett. 60:273; Palva *et al.* (1982) Proc. Natl. Acad. Sci. USA 79:5582; EP Publ. Nos. 036 259 and 063 953; PCT WO 84/04541, *Bacillus*), (Miller *et al.* (1988) Proc. Natl. Acad. Sci. 85:856; Wang *et al.* (1990) J. Bacteriol. 172:949, *Campylobacter*), (Cohen *et al.* (1973) Proc. Natl. Acad. Sci. 69:2110; Dower *et al.* (1988) Nucleic Acids Res. 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids in Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) J. Mol. Biol. 53:159; Taketo (1988) Biochim. Biophys. Acta 949:318; Escherichia), (Chassy *et al.* (1987) FEMS Microbiol. Lett. 44:173 *Lactobacillus*); (Fiedler *et al.* (1988) Anal. Biochem 170:38, *Pseudomonas*); (Augustin *et al.* (1990) FEMS Microbiol. Lett. 66:203, *Staphylococcus*), (Barany *et al.* (1980) J. Bacteriol. 144:698; Harlander (1987) "Transformation of *Streptococcus lactis* by electroporation," in Streptococcal Genetics (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) Infec. Immun. 32:1295; Powell *et al.* (1988) Appl. Environ. Microbiol. 54:655; Somkuti *et al.* (1987) Proc. 4th Evr. Cong. Biotechnology 1:412, *Streptococcus*).

Transformation methods for yeast hosts are well-known in the art, and typically include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Electroporation is another means for transforming yeast hosts. See for

example, Methods in Enzymology, Volume 194, 1991, "Guide to Yeast Genetics and Molecular Biology." Transformation procedures usually vary with the yeast species to be transformed. See *e.g.*, (Kurtz *et al.* (1986) Mol. Cell. Biol. 6:142; Kunze *et al.* (1985) J. Basic Microbiol. 25:141; *Candida*); (Gleeson *et al.* (1986) J. Gen. Microbiol. 132:3459; Roggenkamp *et al.* (1986) Mol. Gen. Genet. 202:302; *Hansenula*); (Das *et al.* (1984) J. Bacteriol. 158:1165; De Louvencourt *et al.* (1983) J. Bacteriol. 154:1165; Van den Berg *et al.* (1990) Bio/Technology 8:135; *Kluyveromyces*); (Cregg *et al.* (1985) Mol. Cell. Biol. 5:3376; Kunze *et al.* (1985) J. Basic Microbiol. 25:141; U.S. Patent Nos. 4,837,148 and 4,929,555; *Pichia*); (Hinnen *et al.* (1978) Proc. Natl. Acad. Sci. USA 75;1929; Ito *et al.* (1983) J. Bacteriol. 153:163 *Saccharomyces*); (Beach and Nurse (1981) Nature 300:706; *Schizosaccharomyces*); (Davidow *et al.* (1985) Curr. Genet. 10:39; Gaillardin *et al.* (1985) Curr. Genet. 10:49; *Yarrowia*).

Methods for introducing heterologous polynucleotides into mammalian cells are known in the art and include viral infection, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

The method for construction of an expression vector for transformation of insect cells for expression of recombinant herein is slightly different than that generally applicable to the construction of a bacterial expression vector, a yeast expression vector, or a mammalian expression vector. In an embodiment of the present invention, a baculovirus vector is constructed in accordance with techniques that are known in the art, for example, as described in Kitts *et al.*, BioTechniques 14: 810-817 (1993), Smith *et al.*, Mol. Cell. Biol. 3: 2156 (1983), and Luckow and Summer, Virol. 17: 31 (1989). In one embodiment of the present invention, a baculovirus expression vector is constructed substantially in accordance to Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Moreover, materials and methods for baculovirus/insect cell expression systems are commercially available in kit form, for example, the MaxBac® kit from Invitrogen (San Diego, CA).

Also, methods for introducing heterologous DNA into an insect host cell are known in the art. For example, an insect cell can be infected with a virus containing the

desired coding sequence. When the virus is replicating in the infected cell, the desired polypeptide will be expressed if operably linked to a suitable promoter. A variety of suitable insect cells and viruses are known and include following without limitation.

Insect cells from any order of the Class Insecta can be grown in the media of this invention. The orders Diptera and Lepidoptera are preferred. Example of insect species are listed in Weiss *et al.*, "Cell Culture Methods for Large-Scale Propagation of Baculoviruses," in Granados *et al.* (eds.), The Biology of Baculoviruses: Vol. II Practical Application for Insect Control, pp. 63-87 at p. 64 (1987). Insect cell lines derived from the following insects are exemplary: *Carpocapsa pomeonella* (preferably, cell line CP-128); *Trichoplusia ni* (preferably, cell line TN-368); *Autograph californica*; *Spodoptera frugiperda* (preferably, cell line Sf9); *Lymantria dispar*; *Mamestra brassicae*; *Aedes albopictus*; *Orgyia pseudotsugata*; *Neodiprio sertifer*; *Aedes aegypti*; *Antheraea eucalypti*; *Gnorimoschema operceullela*; *Galleria mellonella*; *Spodoptera littolaris*; *Blatella germanic*; *Drosophila melanogaster*; *Heliothis zea*; *Spodoptera exigua*; *Rachiplusia ou*; *Plodia interpunctella*; *Amsaeta moorei*; *Agrotis c-nigrum*, *Adoxophyes orana*; *Agrotis segetum*; *Bombyx mori*; *Hyponomeuta malinellu;*, *Colias eurytheme*; *Anticarsia germmetalia*; *Apanteles melanoscelu*; *Arctia caja*; and *Porthetria dispar*. Preferred insect cell lines are from *Spodoptera frugiperda*, and especially preferred is cell line Sf9. The Sf9 cell line used in the examples herein was obtained from Max D. Summers (Texas A & M University, College Station, Texas, 77843, U.S.A.) Other *S. frugiperda* cell lines, such as IPL-Sf-21AE III, are described in Vaughn *et al.*, In Vitro 13: 213-217 (1977).

The insect cell lines of this invention are suitable for the reproduction of numerous insect-pathogenic viruses such as parvoviruses, pox viruses, baculoviruses and rhabdcoviruses, of which nucleopolyhedrosis viruses (NPV) and granulosis viruses (GV) from the group of baculoviruses are preferred. Further preferred are NPV viruses such as those from Autographa spp., Spodoptera spp., Trichoplusia spp., Rachiplusia spp., Gallerai spp., and Lymantria spp. More preferred are baculovirus strain *Autographa californica* NPV (AcNPV), *Rachiplusia ou* NPV, *Galleria mellonella* NPV, and any plaque purified strains of AcNPV, such as E2, R9, S1, M3, characterized and described by Smith *et al.*, J Virol 30: 828-838 (1979); Smith *et al.*, J Virol 33: 311-319 (1980); and Smith *et al.*, Virol 89: 517-527 (1978).

Typically, insect cells *Spodoptera frugiperda* type 9 (SF9) are infected with baculovirus strain *Autographa californica* NPV (AcNPV) containing the desired coding sequence. Such a baculovirus is produced by homologous recombination between a transfer vector containing the coding sequence and baculovirus sequences and a genomic baculovirus DNA. Preferably, the genomic baculovirus DNA is linearized and contains a dysfunctional essential gene. The transfer vector, preferably, contains the nucleotide sequences needed to restore the dysfunctional gene and a baculovirus polyhedrin promoter and terminator operably linked to the desired coding sequence. (See Kitts *et al.*, BioTechniques 14(5): 810-817 (1993).

The transfer vector and linearized baculovirus genome are transfected into SF9 insect cells, and the resulting viruses probably containing the desired coding sequence. Without a functional essential gene the baculovirus genome cannot produce a viable virus. Thus, the viable viruses from the transfection most likely contain the desired coding sequence and the needed essential gene sequences from the transfer vector. Further, lack of occlusion bodies in the infected cells are another verification that the desired coding sequence was incorporated into the baculovirus genome.

The essential gene and the polyhedrin gene flank each other in the baculovirus genome. The coding sequence in the transfer vector is flanked at its 5' with the essential gene sequences and the polyhedrin promoter and at its 3' with the polyhedrin terminator. Thus, when the desired recombination event occurs the desired coding sequence displaces the baculovirus polyhedrin gene. Such baculoviruses without a polyhedrin gene will not produce occlusion bodies in the infected cells. Of course, another means for determining if coding sequence was incorporated into the baculovirus genome is to sequence the recombinant baculovirus genomic DNA. Alternatively, expression of the desired polypeptide by cells infected with the recombinant baculovirus is another verification means.

Assays for Determining the IL8R1 Binding

Receptor binding assays herein may utilize cells that naturally produce the IL8R1 receptor, such as human neutrophils. Alternatively, a polynucleotide encoding a native IL8R1 can be introduced into a cell to produce a IL8R1. For the assay, either whole

cells or membranes can be used to determine receptor binding. Typically, the assay for receptor binding is performed by determining if the present polypeptide can compete with radioactive, native IL8 or IL8R1 binding compounds for binding to IL8R1. The less radioactivity measured the less native IL8 bound to the receptor. See Sakurai *et al.*, EP 480 381 and Adachi *et al.*, FEBS Lett 311(2): 179-183 (1992) for examples of receptor binding assays.

The IL8R1 binding can also be measured utilizing signal transduction assays. The IL8R1 binding compounds which inhibit IL8 activity can compete with native IL8 to modulate signal transduction. Typical signal transduction assays measure $Ca2^+$, $IP_3$, and DAG levels as described herein.

Most cellular $Ca2^+$ ions are sequestered in the mitochondria, endoplasmic reticulum, and other cytoplasmic vesicles, but binding of IL8 to IL8R1 will trigger the increase of free $Ca^{2+}$ ions in the cytoplasm. With fluorescent dyes, such as *fura*-2, the concentration of free $Ca^{2+}$ can be monitored. The ester of fura-2 is added to the media of the host cells expressing $ETB_1$ receptor polypeptides. The ester of fura-2 is lipophilic and diffuses across the membrane. Once inside the cell, the fura-2 ester is hydrolyzed by cytosolic esterases to its non-lipophilic form, and then the dye cannot diffuse back out of the cell. The non-lipophilic form of fura-2 will fluoresce when it binds to the free $Ca^{2+}$ ions, which are released after binding of a ligand to IL8R1. The fluorescence can be measured without lysing the cells at an excitation spectrum of 340 nm or 380 nm and at fluorescence spectrum of 500 nm.

The rise of free cytosolic $Ca^{2+}$ concentrations is preceded by the hydrolysis of phosphatidylinositol 4,5-bisphosphate. Hydrolysis of this phospholipid by the plasma-membrane enzyme phospholipase C yields 1,2-diacylglycerol (DAG), which remains in the membrane, and the water-soluble inositol 1,4,5-triphosphate ($IP_3$). Binding of endothelin or endothelin agonists will increase the concentration of DAG and $IP_3$. Thus, signal transduction activity can be measured by monitoring the concentration of these hydrolysis products.

To measure the $IP_3$ concentrations, radioactively labelled $^3$H-inositol is added to the media of host cells expressing IL8R1. The $^3$H-inositol taken up by the cells, and after stimulation of the cells with IL8, the resulting inositol triphosphate is separated from the mono and di-phosphate forms and measured. Alternatively, Amersham provides

an inositol 1,4,5-triphosphate assay system. With this system Amersham provides tritylated inositol 1,4,5-triphosphate and a receptor capable of distinguishing the radioactive inositol from other inositol phosphates. With these reagents an effective and accurate competition assay can be performed to determine the inositol triphosphate levels.

C. Examples

The examples presented below are proved as a further guide to the practitioner of ordinary skill in the art, and are not to be construed as limiting the invention in any way.

(i) Materials for the following examples:

Recombinant human IL8, GROα (GRO/MGSA), and $^{125}$I-IL8 were prepared as described in Hammond, M.E.W. *et al.*, J. Immunol. 155: 1428-1433 (1995).

(ii) Construction of Polypeptides SEQ ID NO:2 to SEQ ID NO:15

All constructs were generated by PCR according to Shyamala *et al.*, Methods in Molecular Biology 15: 339-348 (1993) with the exception that vent Polymerase was used in the place of Taq Polymerase. The DNA template was either the native IL8 or *il8syn*. *il8syn* was constructed as described in Miller *et al.*, Protein Expression. Purif. 6: 357-362 (1995). The IL8 sequence is also described in Aschauer *et al.*, PCT WO89/04836 and Gregory *et al.*, Proc. Natl. Acad. Sci. USA 85: 9199-9203 (1988).

Amino acid substitutions were introduced according to Shyamala et al., Gene (Amst.) 97: 1-6 (1991) by overlap PCR using the sense and antisense mutated primers in combination with appropriate end primers as listed in the table below:

| | |
|---|---|
| *Y13L* | AAGACACTGTCGGGACCTTTC |
| *S14Q* | AAGACATATCAGAAACCTTTC |
| *F21N* | CACCCCAAAAACATCAAAGAA |
| *V41F* | GAAATTATTTTCAAACTTTCT |
| *D45R* | AAACTTTCTCGCGGAAGAGAG |
| *R47K* | GATGGAAAGGAGCTCTGTCT |
| *L49A* | GATGGAAGAGAGGCTTGTCT |
| *L49F* | GGAAGAGAGTCGTGTCTGGAC |
| *L49S* | GGAAGAGAGTTCTGTCTGGAC |
| *Y13L,S14Q* | AAGACACTGCAGAAACCTTTC |
| *R47K,D52N* | (See note below) |
| *E48K,L49A* | GATGGAAGAAAAGCTTGTCT |
| *E48K,D52N* | GATGGAAGAAAACTCTGTCT<br>Also, see note below. |

| | |
|---|---|
| *R47K,E48K,D52N* | GATGGAAAGAAACTCTGTCT<br>Also, see note below |

The mutations in combination with D52N were obtained by amplifying respective mutated DNA templates with the primers for D52N mutation.

Mutations were generated with the following 5' sense and 3' antisense end primers

5' end sense

GAAGGGGTACCCTTGGATAAGAGAAGTGCTAAAGAACTTAGATGTCAA

3' end antisense

AGACCGCTCGAGCTATTATGAATTCTCAGCC.

In addition to these end primers, sense primers were used, the sequence of which is shown in the table above. Also, antisense primers were utilized, the sequence of which is the reverse-complement of the sequences in table above.

(iii) Protein Expression and Purification

Expression cassettes for yeast secretion were transferred as *Bam*HI restriction fragments into vector pAB24 and introduced into Saccharomyces cerevisae strain MB2-1 by electroporation. Vector pAB24 is described Brake *et al.*, Brake *et al.*, Methods Enzymol. 185: 408-421 (1990) and EP 324 274.

Chimeric and mutant chemokines were purified from 50-200 mL of yeast culture broth by batch adsorption on S-Sepharose, Fast Flow (Pharmacia Biotech Inc., Uppsala, Sweden) after adjustment to pH5.5 with 50 mM sodium acetate and eluted in 20 mM HEPES, pH 8.3 1 M NaCl to a final concentration of 0.2-2 mg.mL.

SDS-polyacrylamide gel electrophoresis on 18% Tris/glycine gels indicated 80-95% purity. Protein conentrations were estimated by Coomassie-stained polyacrylamide gels and by BCA protein assays. Amino acid composition and amino-terminal sequencing were performed on selected proteins and agreed with predicted protein sequences.

Binding Assays-

Competitive binding assays for chimeric proteins were performed on CHO-IL8R1and CHO-IL8R2 essentially as described in Hammond *et al.*, J. Immunol. 155: 1428-1433 (1995).and Tekamp-Olson et al., PCT WO95/07934. Assays were performed in triplicate and data were analyzed by GraFit version 2.0, Erithacus Software, Ltd., Staines United Kingdom, written by Leatherbarrow.

Chemotaxis Assays

Assays were performed in triplicate on freshly isolated human neutrophils as described in Hammond *et al., supra.* chemotaxis to f-Met-Leu-Phe (100 nM) was measured as a positive control for each experiment.

Deposit Information

The following materials wre deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852:

S. cerevisae MB2-1 (pYMIP540) deposited 20 June 1990, ATCC no. 74002.

These materials were deposited under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-Organisms for Purposes of Patent Procedure. These deposits are provided as a convenience to those of skill in the art, and do not represent an admission that a deposit is required under 35 USC Section 112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with the written description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Wernette-Hammond, Mary Ellen
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Siani, Michael
Blaney, Jeff
Tekamp-Olson, Patricia

(ii) TITLE OF INVENTION: Polypeptides with Interleukin 8 Receptor Binding

(iii) NUMBER OF SEQUENCES: 30

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(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94608

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1               5                  10                  15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
            20                  25                  30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
        35                  40                  45

Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
    50                  55                  60
```

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Lys Glu
35 40 45

Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
35 40 45

Ala Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Lys
35 40 45

Ala Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Lys
35 40 45

Leu Cys Leu Asn Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro

```
                    20                  25                  30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Lys Lys
        35                  40                  45

Leu Cys Leu Asn Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
    50                  55                  60

Phe Leu Lys Arg Ala Glu Asn Ser
65                  70
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1               5                   10                  15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
            20                  25                  30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Lys Glu
        35                  40                  45

Leu Cys Leu Asn Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
    50                  55                  60

Phe Leu Lys Arg Ala Glu Asn Ser
65                  70
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Leu Ser Lys Pro
1               5                   10                  15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
            20                  25                  30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
        35                  40                  45

Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
    50                  55                  60

Phe Leu Lys Arg Ala Glu Asn Ser
65                  70
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Gln Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
35 40 45

Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Phe Lys Leu Ser Asp Gly Arg Glu
35 40 45

Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Arg Gly Arg Glu
35 40 45

Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
35 40 45

Phe Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
35 40 45

Ser Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
1 5 10 15

Phe His Pro Lys Asn Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
35 40 45

Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Leu Gln Lys Pro
1 5 10 15

Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
20 25 30

His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
35 40 45

Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
50 55 60

Phe Leu Lys Arg Ala Glu Asn Ser
65 70

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AAGACACTGT CGGGACCTTT C 21

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AAGACATATC AGAAACCTTT C 21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CACCCCAAAA ACATCAAAGA A 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAAATTATTT TCAAACTTTC T 21

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AAACTTTCTC GCGGAAGAGA G 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GATGGAAAGG AGCTCTGTCT 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GATGGAAGAG AGGCTTGTCT 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGAAGAGAGT CGTGTCTGGA C 21

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGAAGAGAGT TCTGTCTGGA C 21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAGACACTGC AGAAACCTTT C 21

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATGGAAGAA AAGCTTGTCT 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GATGGAAGAA AACTCTGTCT 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GATGGAAAGA AACTCTGTCT 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GAAGGGGTAC CCTTGGATAA GAGAAGTGCT AAAGAACTTA GATGTCAA 48

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGACCGCTCG AGCTATTATG AATTCTCAGC C 31

WHAT IS CLAIMED:

1. A polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) R47K: (SEQ ID NO:2):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Lys Glu
Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(b) L49A (SEQ ID NO:3):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
Ala Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(c) E48K,L49A (SEQ ID NO:4):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Lys
Ala Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(d) E48K, D52N (SEQ ID NO:5):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Lys
Leu Cys Leu Asn Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(e) R47K, E48K, D52N (SEQ ID NO:6):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Lys Lys
Leu Cys Leu Asn Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(f) R47K, D52N (SEQ ID NO:7):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Lys Glu
Leu Cys Leu Asn Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(g) Y13L (SEQ ID NO:8):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Leu Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(h) S14Q (SEQ ID NO:9):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Gln Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(i) V41F (SEQ ID NO:10):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Phe Lys Leu Ser Asp Gly Arg Glu
Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(j) D45R (SEQ ID NO:11):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Arg Gly Arg Glu
Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser

(k) L49F (SEQ ID NO:12):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
Phe Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(l) L49S (SEQ ID NO:13):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
Ser Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser;

(m) F21N (SEQ ID NO:14):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro
Phe His Pro Lys Asn Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser; and

(n) Y13L, S14Q (SEQ ID NO:15):

Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Leu Gln Lys Pro
Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro
His Cys Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu
Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys
Phe Leu Lys Arg Ala Glu Asn Ser.

2. A polypeptide comprising an amino acid sequence which is a mutant of a reference sequence selected from the group consisting of:

SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.

3. The polypeptide of Claim 2, wherein said amino acid sequence exhibits at least 80% sequence identity to the reference sequence.

4. A polypeptide comprising an amino acid sequence which is a fragment of a reference sequence selected from the group consisting of:

SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.

5. The polypeptide of Claim 4, wherein said amino acid sequence exhibits at least 80% sequence identity to the reference sequence.

6. A polypeptide comprising an amino acid sequence which is a fusion of a reference sequence selected from the group consisting of:

SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.

7. The polypeptide of Claim 6, wherein said amino acid sequence exhibits at least 80% sequence identity to the reference sequence.

8. A polynucleotide comprising a sequence coding an amino acid sequence selected from the group consisting of:

SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.

9. A polynucleotide of Claim 8, further comprising a promoter which is operably linked to said coding sequence.

10. A host cell comprising the polynucleotide of Claim 9.

11. A method of producing a polypeptide capable of modulating an IL8 receptor-mediated biological response comprising:

(a) providing a host cell comprising a polynucleotide that comprises a sequence coding an amino acid sequence selected from the group consisting of:

SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.

(b) culturing the host cell under conditions that induce expression of the polypeptide.

12. A method of modulating an IL8 receptor-mediated biological response by contacting cells capable of an IL8 receptor-mediated response with an effective amount of polypeptide comprising an amino acid sequence selected from the group consisting of:

SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.

13. A method of inhibiting IL8 receptor binding by contacting cells producing IL8 receptor polypeptides with an inhibiting amount of a polypeptide comprising an amino acid sequence selected from the group consisting of:

SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.